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Correlation between the dielectric properties and the physicochemical characteristics and proximate composition of whole, semi-skimmed and skimmed sheep milk using chemometric tools

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A B S T R A C T

The influence of temperature, frequency, composition and physicochemical characteristics of sheep milk on its dielectric properties was evaluated as well as the performance of different chemometric methods to predict the dielectric properties and classify the type of sheep milk (whole, semi-skimmed and skimmed). Of the chemometric methods evaluated, artificial neural network exhibited the best performance for prediction of the dielectric properties, while sensitivity analysis showed temperature, electrical conductivity, and fat and calcium content as variables with the most impact. All pattern recognition techniques showed 100% for recognition and prediction ability to classify correctly the type of milk. Although the approach used in this study is limited to the specific operating conditions and sheep milk studied, chemometric methods have proven to be promising tools because of accuracy and suitability for both prediction of the dielectric properties of sheep milk and monitoring quality control parameters of milk and dairy products.

1. Introduction

Sheep milk production plays an important economic role in many countries, especially those bordering the Mediterranean Sea, Middle East, sub-Saharan Africa and East and Southeast Asia (Balthazar et al., 2017a). Sheep milk has been used as a raw material for production of different dairy foods such as, cheese (Albenzio et al., 2013a,b; 2015a,b), yoghurt (Balthazar et al., 2015a,b; 2016) and ice cream (Balthazar et al., 2017b,c). Sheep dairy products have gained market size due to their superior product quality, high yield, and nutritional value when compared with milk of other ruminant species, probably due to the higher content of protein (5.6%), lipids (6.4%), minerals (0.9%), and essential vitamins (Kalyankar, Sarode, Khedkar, Deosarkar, & Pawshe, 2016).

Conventional thermal processes have been the most economically feasible, common and widely used techniques to inactivate microorganisms and undesirable enzymes in the dairy industry (Amaral et al., 2017; Cappato et al., 2017; Coutinho et al., 2018). Nevertheless, thermal processes have low thermal efficiency and negatively affect sensory and nutritional quality attributes (Xu et al., 2016). In addition, another common problem with conventional heating is the fouling phenomenon in the heat exchanger, over time, due to high surface temperature. Fouling reduces heat transfer, thus affecting food quality due to overheating or insufficient heating, and causes changes in flavour due to decomposition of the fouled material (Tewari, 2007; Zhu, Guo, & Jia, 2014; Zhu, Kuznetsov, & Sandeep, 2007).

Microwave heating results from the ability of dielectric materials to absorb microwave energy and to convert it to heat (Chandrasekaran, Ramanathan, & Basak, 2013). This conversion is achieved by dipole rotation and ionic conduction mechanisms, which increases kinetic energy and internal molecular friction, thus increasing the material temperature (Martins et al., 2019; Stratakos,

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Delgado-Pando, Linton, Patterson, & Koidis, 2016). Microwave heating allows a more efficient heating process with no fouling, higher penetrative power, faster heating rates, and shorter processing times when compared with conventional heating methods, resulting in a product with better sensorial, nutritional, and functional properties (Siguemoto & Gut, 2016).

Interaction between the microwave field and the food product is determined by the dielectric properties, which are represented by the relative electrical permittivity (ε', also known as dielectric constant), which describes the capacity of the material to store electrical energy, and the dielectric loss factor (ε"), which describes the ability of the material to dissipate electrical energy into heat (Salazar-González, Martín-González, López-Malo, & Sosa-Morales, 2012; Sosa-Morales, Valerio-Junco, Lopez-Malo, & Garcia, 2010). They are the real and imaginary parts, respectively, of the relative complex permittivity ($\varepsilon^* = \varepsilon' - j\varepsilon''$). The amount of thermal energy converted into heat in food is proportional to the dielectric loss factor (Tang, 2005). At frequencies of 915 and 2450 MHz, the dipole rotation (ε_σ'') and ionic conduction (ε_d'') are the predominant mechanisms affecting the dielectric loss factor (Ryynänen, 1995), which can be described as $\varepsilon'' = \varepsilon_d'' + \varepsilon_\sigma'' = \varepsilon_d'' + \sigma/2\pi f \varepsilon_0$, wherein σ is the electrical conductivity (mS cm^{-1}), f is the electric field frequency (Hz), and ε_0 is the permittivity of free space (8.854×10^{-12} F m^{-1}). These properties govern the microwave penetration into the food and local power absorption rates, thus improving the heating performance of the product (Martín-Esparza, Martínez-Navarrete, Chiralt, & Fito, 2006), being mostly affected by the operating parameters (temperature and electric field frequency) and the characteristics of the material (chemical composition, salt content, pH, water activity, etc.; Franco, Yamamoto, Tadini, & Gut, 2015).

A combination of instrumental techniques and multivariate data analysis has an important role in control and monitoring of the different steps in food processing. The ability to predict multiple parameters has revolutionised many applications currently in use by the food industry, as well as allowing for the development of new applications (Roberts & Cozzolino, 2016). Chemometric techniques, such as PLS, MLR, ANN, principal component analysis (PCA), and LDA have been applied for many different purposes, including calibration, prediction of composition, geographical origin, regionality, varietal, monitor contamination, authenticity and adulteration, varietal identification, among others (Granato et al., 2018). In the near future, these applications will provide methods to screen the composition and variation of raw materials and foods in real time, thus identifying undesirable issues or faults, by providing a rapid means of analysis (Roberts & Cozzolino, 2016).

Although dielectric properties have been determined for different dairy foods (Nelson & Trabelsi, 2012; Nunes, Bohigas, & Tejada, 2006; Zhu et al., 2014; Zhu, Guo, Jia, & Kang, 2015b; Zhu, Guo, & Liang, 2015a), its use to control quality parameters in the dairy industry, in particular the sheep milk industry, has not been reported in the literature. The objectives of this study were to: (i) determine the dielectric properties of whole, semi-skimmed, and skimmed raw sheep milk in a range of frequency and temperature adequate for microwave heating; (ii) investigate the effect of temperature, frequency, composition, and physicochemical characteristics of sheep milk on its dielectric properties; (iii) evaluate mathematical models and chemometric tools to predict dielectric properties and classify type of milk.

2. Material and methods

2.1. Sheep milk processing

Raw sheep milk [25.67 g fat 100 g^{-1} dry basis (d.b.)] was obtained from a Lacaune ewe flock, located in a mountainous region of Rio de Janeiro State, Brazil, being semi-skimmed and skimmed approximately to 15.58 and 2.37 g fat 100 g^{-1} d.b. by centrifugation (Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany) at 1792 $\times g$ at 4 °C for 5 and 12 min, respectively. Whole, semi-skimmed and skimmed raw sheep milk were heat-treated (72–75 °C, 15 s) using a plate heat exchanger (BCISMINI, Equilati, São Paulo, Brazil). The sheep milk processing was carried out at the Dairy Laboratory Pilot Plant (Núcleo Avançado de Educação em Tecnologia de Alimentos e Gestão de Cooperativismo – NATA) in São Gonçalo, Rio de Janeiro, Brazil. Sheep milk samples were packed in 1000-mL high-density polyethylene bottles and stored in a plasma freezer (FANEM MOD 349FV, São Paulo, Brazil) at −30 °C.

2.2. Physiochemical analyses

Sheep milk samples were characterised for colour attributes, total titratable acidity (TTA), pH, and water activity (a_w), according to the methodologies of AOAC (2012). TTA was measured at the titration endpoint of pH 8.2 (0.0997 N NaOH) using a pH-Stat PHM-290 (Radiometer, Copenhagen, Denmark), and expressed as a percentage of lactic acid. The pH of the samples was determined using the same apparatus. Water activity at 25.0 ± 0.5 °C was measured with an AquaLab 3TE (Decagon Devices Inc., Pullman, WA, USA). Colour was determined by Hunter Lab (Riston, VA, USA), using CIE*Lab* scale (L^*, a^* and b^*), RSEX calibration with D65 illuminant.

2.3. Proximate composition

Proximate composition (moisture, ash, fat, protein, and carbohydrates, g 100 g^{-1}) was determined using conventional methods (AOAC, 2012). Moisture was determined by drying 10 g sample at 70 °C for 72 h in a vacuum oven to constant weight. Fat was quantified by the Gerber method. Protein was determined by the Kjeldahl method, by multiplying the nitrogen content by the conversion factor 6.38. Ash was determined by incineration of 5 g of previously dry sample at 550 °C for 48 h in a muffle furnace to constant weight. Carbohydrates content was determined by difference. Results were reported on dry basis, except moisture.

2.4. Calcium content

The calcium content was determined by inductively coupled plasma (ICP) optical emission spectrometry (Spectro Analytical Instruments, Kleve, Germany) according to Moreno-Rojas, Pozo-Lora, Zurera-Cosano, and Amaro-Lopez (1994), with certain modifications. Analytical curves were built using calcium standards. Ten grams of each sample was acid hydrolysed for 16 h at 120 ± 2 °C using 2 mL nitric-perchloric acid solution (2:1, v/v). Samples were then heated in a digestion block (Technal, São Paulo, Brazil) in a fume hood at slow boil to 100 ± 2 °C for 1 h and kept for 2 h at 170 ± 2 °C. After cooling to room temperature, 2 mL of nitric-perchloric acid were added to each tube and heated for 4 h at 170 ± 2 °C in a digestion block and then cooled to room temperature.

2.5. Electrical conductivity

Electrical conductivity (σ) of the samples was determined with conductivity meter YSI3200 and cell YSI3252 (YSI, Yellow Springs, OH, USA) over a temperature ranging from 5 to 90 °C. Temperature of the sample was controlled using a thermostatic oil bath TC550 (Brookfield, Middleboro, MA, USA) and a thermocouple (Mileto, São Paulo, Brazil). For each experiment, three conductivity readings were made at the selected temperature before raising the temperature to the next level.

2.6. Dielectric properties measurement

A vector network analyser (E5061B, Agilent Technologies, Malaysia) connected to an open-ended coaxial-line probe (85093C, Agilent Technologies) with a coaxial cable (N6314A, Agilent Technologies) was used to measure the dielectric properties of sheep milk samples. The network analyser measured the reflection coefficient at the probe-sample interface. The software of the dielectric probe kit (85070E, Agilent Technologies) was used to calculate the dielectric properties (relative electrical permittivity, ε'; and dielectric loss factor, ε"), and an electronic calibration module (85093C, Agilent Technologies) was used to minimise interferences. Calibration was performed using three standards: open circuit (air), short circuit, and deionised water at room temperature. Dielectric measurements of the sample were performed at different frequencies (500–3000 MHz) and temperatures (5–90 °C). For that, 150 mL milk was placed in an Erlenmeyer flask and immersed in a thermostatic oil bath TC-550 (Brookfield). Measurements were made after the temperature of the sample reached the desired temperature, which was monitored with a thermocouple (Mileto) with a tolerance of ±1 °C. For each experiment, seven readings (frequency sweeps) were made at the selected temperature before raising the temperature to the next level.

2.7. Power penetration depth

Power penetration depth (d_p), is a useful parameter for microwave heating applications, and the best frequency should be selected for a rapid and uniform thermal treatment. Penetration depth of the microwaves is defined as the depth in which the power is reduced to $1/e = 36.8\%$ (Euler number: $e = 2.7183$) of the incident power at the surface of the material. It is an important parameter in evaluating heating uniformity and designing electromagnetic heating equipment. Penetration depth is a function of wavelength and dielectric properties of the material, and it can be calculated according to Equation (1) (Risman, 1991):

$$d_p = \frac{c}{2\pi f\sqrt{2\varepsilon'\left[\sqrt{1+\left(\frac{\varepsilon''}{\varepsilon'}\right)^2}-1\right]}} \quad (1)$$

where c is the speed of light in free space (2.9979×10^8 m s^{-1}), and f is the frequency of the electromagnetic wave. The penetration depths at frequencies of 915 and 2450 MHz were calculated from the readings of the dielectric properties at each temperature.

2.8. Data analyses

2.8.1. Prediction of temperature-dependence by polynomial regression

First, polynomial correlations were adjusted by modelling possible temperature-dependence of electrical conductivity (σ, mS cm^{-1}), relative electrical permittivity (ε'), dielectric loss factor (ε"), power penetration depth (d_p, mm), contribution of ionic ($C_\sigma" = \varepsilon_\sigma"/\varepsilon"$) and dipole rotation ($C_d" = \varepsilon_d"/\varepsilon"$) mechanisms on the dielectric loss factor, respectively, and loss tangent (tanδ = ε"/ε') for the commercial frequencies of 915 and 2450 MHz. Model fitting was done using the polynomial regression tool of the software Statgraphics Centurion XV (Statpoint, Warrenton, VA, USA) and the smaller polynomial coefficient was used to provide a good fit. The polynomial order and correlation coefficients were chosen based on the coefficient of determination (R^2), the number of parameters, and significance of the parameter ($p < 0.05$) (Siguemoto & Gut, 2016; Yaghmaee & Durance, 2001).

2.8.2. Prediction of the multivariable effect on the dielectric properties

Multiple linear regression (MLR), partial least squares regression (PLSR) and artificial neural network (ANN) were used to model the dielectric properties of the sheep milk samples (ε', ε", d_p, C_σ", C_d" and tanδ) as a function of temperature (T), electrical conductivity (σ), physicochemical properties (a_w, TTA and pH), colour attributes (L^*, a^* and b^*), and composition (moisture, ash, protein, fat, carbohydrates and calcium content) of the sheep milk. Prior to chemometric analysis, each variable was mean-centering autoscaled according to its standard deviation to obtain relativised data. Two different datasets were evaluated separately for 915 and 2450 MHz, both comprised by 90 lines (samples) and 18 columns (variables). MLR was tentatively performed by Statgraphics Centurion XV (Statpoint), attempting to model the relationship between two or more interpretive variables (independent) and a linear response variable (dependent), by fitting an equation for the observed data. PLSR is a multilinear regression model that reduces the number of observable variables and extracts several principal components (PCs) to describe a maximum correlation among the observed variables and the predicted variables. PLSR was performed with Pirouette 2.2 (Infometrix, Bothell, WA, USA). ANN was performed with Matlab 7.5 (The MathWorks Inc, Natick, MA, USA) using the Levenberg–Marquardt training algorithm. Multiple ANN topologies were assayed using a multilayer perceptron (MLP) comprised of three layers (input, hidden and output), and employing different numbers of hidden neurons (3–20) and activation functions. All datasets were randomly divided into the training set (70%) and test set (30%). The training set was used to build the model, whereas the test set was used to measure the predictive ability of the model. The best choice for network, topology, and number of neurons in the hidden layer was obtained by trial and error. The stopping criterion was 1000 epochs or the maximum sum of squared errors equal to 0.0001 during training. Models were compared based on their coefficient of determination (R^2) and root mean square error (RMSE).

2.8.3. Pattern recognition of the milk type

Linear discriminant analysis (LDA), soft independent modelling of class analogies (SIMCA), k-nearest neighbours algorithm (k-NN) and ANN were used as pattern recognition techniques to classify the type of milk (whole, semi-skimmed and skimmed) using dielectric properties as independent variables. As previously mentioned, the data were autoscaled to obtain relativised data before chemometrics application. Two different datasets were evaluated for the frequencies 915 and 2450 MHz. Both datasets were comprised by 90 lines (samples) and 7 columns (variables), in which one column corresponds to the dependent variable (the type of milk) and the others corresponding to the independent variables (ε', ε", d_p, C_σ", C_d" and tanδ). The classification rules achieved by the techniques were obtained by randomly dividing each dataset into two parts: the training set (70% of the samples) and a test set (30% of the samples). Such a division allows for a sufficient number of samples in the training set and a representative number of members among the test set. To identify the natural variation among the samples, the variables were not subjected to any pre-processing of data. For the LDA model, the number of latent variables was obtained by leave-one-out cross-validation (ranged from 2 to 5 latent variables). For k-NN, the Euclidean distance was used to calculate the distance between samples, and the optimum number of neighbours (k) to develop the k-NN models ranged from 1 to 5. For SIMCA modelling, the number of principal components (PCs) ranged from 3 to 6. Performance of the models was evaluated by discrimination power, class projections, and misclassification. LDA was performed using XLSTAT Excel plugin 2015.5 (Adinsoft, France). The software Pirouette 2.2 (Infometrix) was used for SIMCA and *k*-NN analyses, and ANN was performed using software Matlab 7.5 (The MathWorks Inc).

2.9. Statistical analysis

All experiments were performed at least in triplicate ($n = 3$). All data are expressed as mean ± standard deviation and were evaluated by analysis of variance (ANOVA) followed by Tukey's honest significant difference (HSD) test at the 95% significance level using the XLSTAT Excel plugin program 2019 (Adinsoft, France) (Granato, Calado, & Jarvis, 2014).

3. Results and discussion

3.1. Physicochemical characteristics

The physicochemical properties of sheep milk are shown in Table 1. All physicochemical properties exhibited a significant ($p < 0.05$) decrease with progressive milk skimming process, except water activity. Water activity (a_w) was high for all samples ($a_w > 0.98$) and varied from 0.993 ± 0.004 (whole milk) to 0.987 ± 0.006 (skimmed milk). TTA values of whole, semi-skimmed, and skimmed milk were 0.2437, 0.3177 and 0.2440 g of lactic acid 100 mL^{-1}, respectively. The pH values slightly differed among the samples, with values of 6.47 (whole milk), 6.38 (semi-skimmed) and 6.30 (skimmed milk). These findings are in accordance with those reported in the literature (Albenzio et al., 2015a; Balthazar et al., 2017a; Hilali, El-Mayda, & Rischkowsky, 2011; Park, Juárez, Ramos, & Haelein, 2007). Colour measurements were expressed by luminosity ($L^* = 0$ black and $L^* = 100$ white), and chromaticity a^* [green (−) and red (+)] and b^* [yellow (−) and blue (+)]. Although all samples exhibited quite similar values for whole ($L^* = 86.8$, $a^* = -2.79$, $b^* = 10.3$), semi-skimmed ($L^* = 87.0$, $a^* = -3.43$, $b^* = 10.7$), and skimmed sheep milk ($L^* = 87.6$, $a^* = -3.55$, $b^* = 10.2$), significant differences were observed among them.

3.2. Proximate composition

The fat contents of whole, semi-skimmed, and skimmed milk were 25.67, 15.58 and 2.37 g 100 g^{-1} d.b., respectively. A significant increase in moisture, ash, protein, and carbohydrates contents was observed with the fat reduction of the samples. Moisture content varied from 75.83 g 100 g^{-1} d.b. (whole milk) to 85.23 g 100 g^{-1} d.b. (skimmed milk) and ash ranged from 4.53 g 100 g^{-1} d.b. (whole milk) to 7.67 g 100 g^{-1} d.b. (skimmed milk). Significant differences were observed for the protein contents, with values of 17.72, 25.15 and 32.97 g 100 g^{-1} d.b. for whole, semi-skimmed and skimmed milk, respectively. Carbohydrates levels were calculated by difference, with values of 52.08, 54.04 and 56.99 g 100 g^{-1} d.b. for whole, semi-skimmed and skimmed milk, respectively, with no significant differences between semi-skimmed and skimmed milk. Calcium content also slightly increased with the decrease in fat content. These findings suggest that the skimming process of sheep milk has a much higher impact on the proximate composition when compared with the physicochemical characteristics. These results are in accordance with those reported in the literature (Balthazar et al., 2017a; Hilali et al., 2011; Park, Juárez, Ramos, & Haenlein, 2007).

3.3. Electrical conductivity

Electrical conductivity is an important parameter for innovative food processes, such as ohmic heating, pulsed electric fields, radio

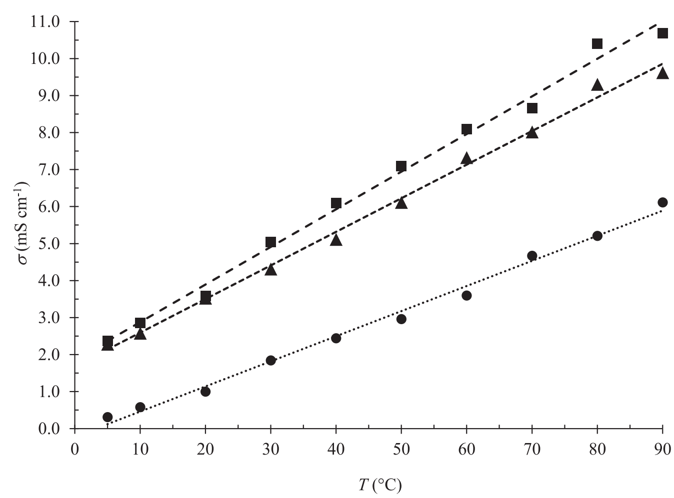

Fig. 1. Electrical conductivity as function of temperature for whole (●), semi-skimmed (▲) and skimmed (■) sheep milk: linearity is indicated by dashed and dotted lines.

Table 1
Physicochemical properties, colour attributes and proximate composition of sheep milk.[a]

Milk characteristics	Sheep milk		
	Whole	Semi-skimmed	Skimmed
Physicochemical properties			
a_w	0.993 ± 0.004^a	0.991 ± 0.001^a	0.987 ± 0.006^a
TTA (g lactic acid 100 mL^{-1})	0.2437 ± 0.0180^b	0.3177 ± 0.0473^a	0.2440 ± 0.0119^b
pH	6.47 ± 0.01^a	6.38 ± 0.01^b	6.3 ± 0.01^c
Colour attributes			
L^*	86.8 ± 0.006^c	87.0 ± 0.001^b	87.6 ± 0.006^a
a^*	−2.79 ± 0.021^a	−3.43 ± 0.006^b	−3.55 ± 0.010^a
b^*	10.3 ± 0.012^b	10.7 ± 0.015^a	10.2 ± 0.006^c
Proximate composition			
Moisture (g 100 g^{-1})	75.83 ± 0.46^c	81.57 ± 1.13^b	85.23 ± 0.20^a
Ash (g 100 g^{-1}, d.b.)	4.53 ± 0.31^c	5.22 ± 0.12^b	7.67 ± 0.04^a
Protein (g 100 g^{-1}, d.b.)	17.72 ± 0.50^c	25.15 ± 1.55^b	32.97 ± 0.59^a
Fat (g 100 g^{-1}, d.b.)	25.67 ± 1.21^a	15.58 ± 1.51^b	2.37 ± 0.75^c
Carbohydrates (g 100 g^{-1}, d.b.)	52.08 ± 1.95^b	54.04 ± 3.15^{ab}	56.99 ± 1.29^a
Calcium (mg 100 g^{-1}, d.b.)	187.2 ± 0.12^c	195.3 ± 0.10^b	196.1 ± 0.11^a

[a] Carbohydrate content was calculated by difference; d.b., dry basis. Data are shown as mean ± standard deviation, values in a row followed by different superscript letters are significantly different ($p < 0.05$).

frequency heating, and microwave heating (Zhang, 2005). For all sheep milk samples, a positive linear correlation was found between the electrical conductivity and temperature (Fig. 1). Ion mobility in a solution is directly linked to its viscosity, which explains the positive effect of temperature on the electrical conductivity. Furthermore, ionic conduction due to the presence of Na^{+}, Ca^{2+}, K^{+}, and Cl^{-} is responsible for most of the electrical conductance of milk. It was observed a slight increase of electrical conductivity with increasing calcium content. However, variation in fat content, fat globule size, and the casein structure, which controls the solubilisation of those ions, also contributed to the overall conductivity. Electrical conductivity of sheep milk decreased as fat content increased, and this deleterious effect is mainly due to fat a poor electric conductor (Binnur & Serap, 2016).

3.4. Dielectric properties

The relative electrical permittivity (ε') and dielectric loss factor (ε") of whole, semi-skimmed and skimmed sheep milk measured from 5 to 90 °C and from 500 to 3000 MHz are shown in Fig. 2A,B, respectively. Frequency and temperature played major roles in the dielectric behaviour of sheep milk samples. The ε' values slightly decreased with increasing frequency for each isotherm studied, which was more pronounced for lower

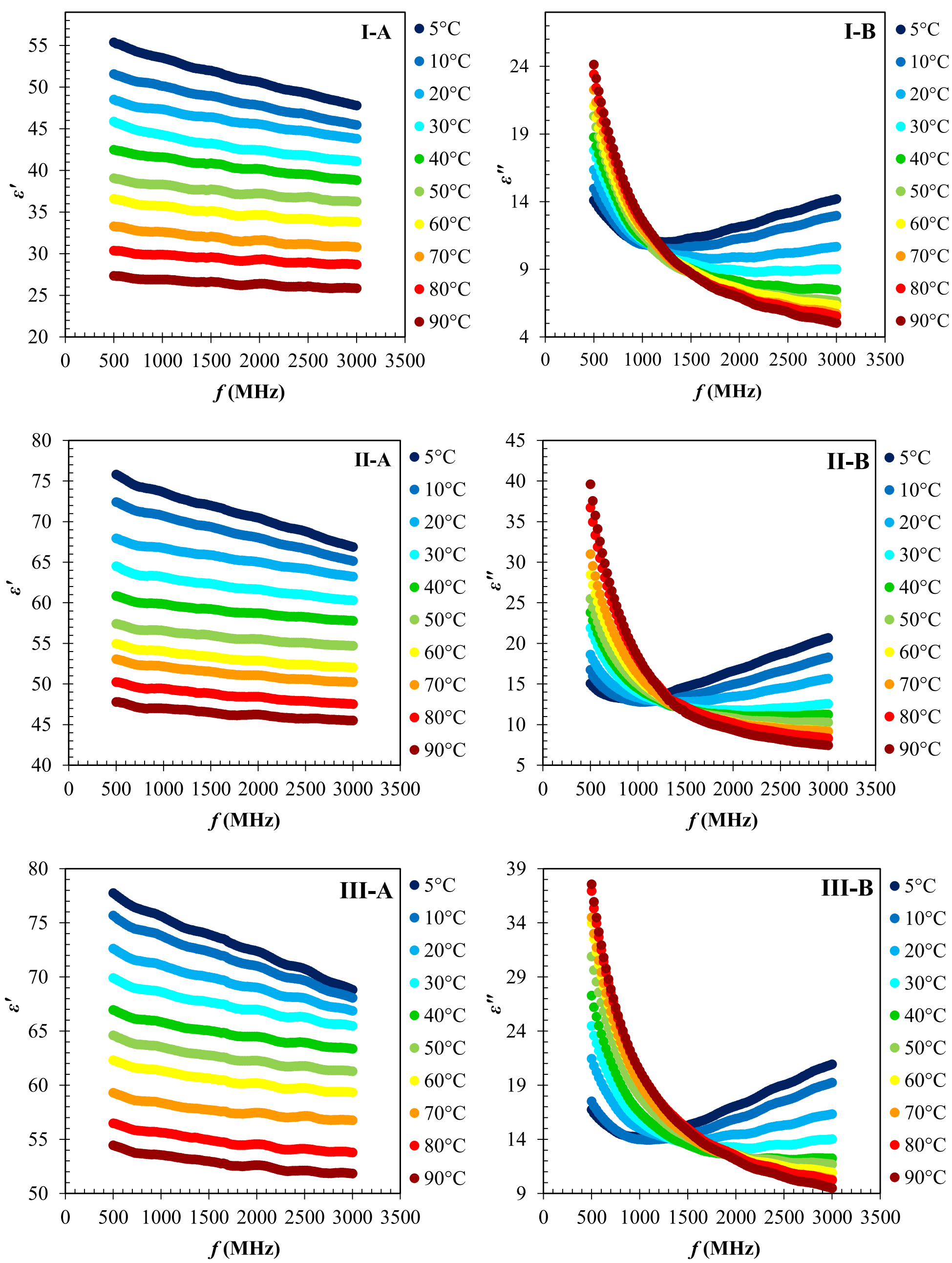

Fig. 2. Relative electrical permittivity (A) and dielectric loss factor (B) of whole (I), semi-skimmed (II) and skimmed (III) sheep milk.

temperatures. The temperature- and frequency-dependent behaviour of the ε' of sheep milk was similar to those of cow milk and goat milk treated over 25–75 °C from 10 to 4500 MHz reported by Zhu et al. (2014). The same tendency was also observed for cow milk over 25–45 °C from 20 to 4500 MHz (Zhu et al., 2015b). The change in dielectric properties with temperature mainly depends on the free and bound water contents in food materials (Gadani, Rana, Bhatnagar, Prajapati, & Vyas, 2012), in which the dielectric polarisation attributable to bound water is much lower than that of free water (Nelson & Trabelsi, 2012). It is interesting to note that temperature has opposite effects on the dielectric loss factor, as a function of frequency. At lower frequencies, an increase in temperature has a positive effect on the loss factor since it increases ion mobility, with a negative effect at higher frequencies, due to an increase in water relaxation frequency, pushing the relaxation peak to higher frequencies (Siguemoto & Gut, 2016). The dielectric properties of the sheep milk in this study followed the same tendencies of other liquid foods, such as cow milk (Zhu et al., 2014), fruit juices (García, Torres, Prieto, & De Blas, 2001; Zhu, Guo, & Wu, 2012), and green coconut water (Franco et al., 2015; Shah, Shah, & Rana, 2015).

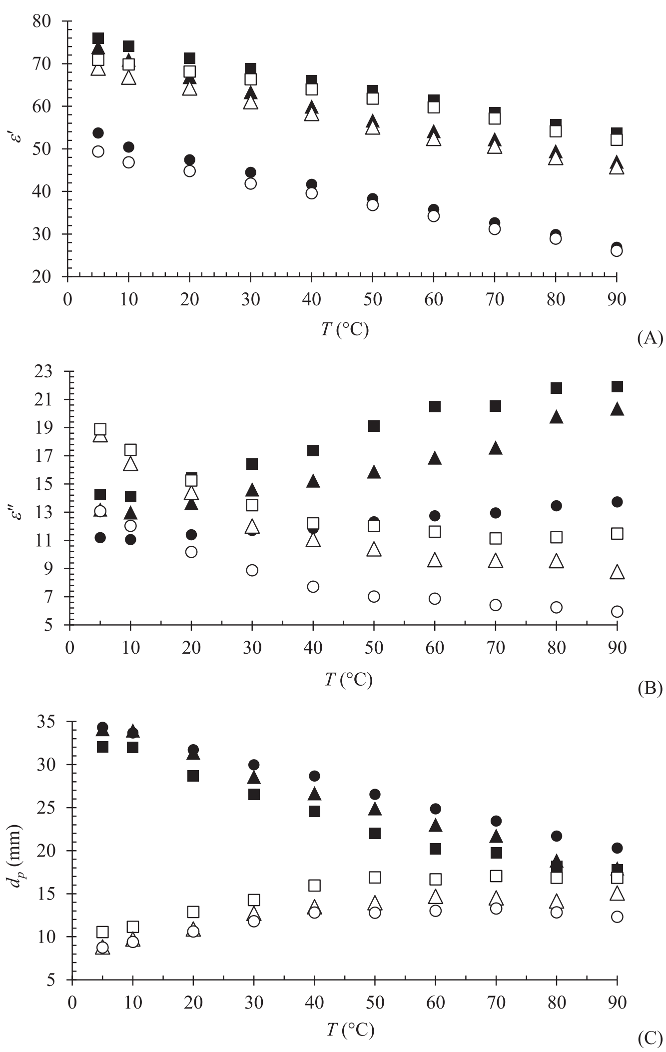

Fig. 3. Relative electric permittivity (A), dielectric loss factor (B) and depth of penetration (d_p) (C) of whole (●, ○), semi-skimmed (▲, △) and skimmed (■, □) sheep milk at 915 MHz (closed symbols) and 2450 MHz (open symbols).

3.5. Commercial frequencies

The present study focused on microwave processing, thus the main results for the commercial frequencies of 915 and 2450 MHz are presented in Fig. 3A (ε'), B (ε") and C (dp). The ε' of the sheep milk decreased almost linearly with temperature for both frequencies (915 and 2450 MHz) for all sheep milk samples. At 915 MHz, the ε' values of whole, semi-skimmed and skimmed milk dropped from 53.7, 73.9 and 75.9 to 26.9, 47.0 and 53.6, respectively. A similar tendency, for slightly lower ε' values, was observed at 2450 MHz. The lowest ε" value was observed for whole milk at both frequencies, which is probably related to lower electrical conductivity due to higher fat content. Although the loss factor decreased with temperature at 2450 MHz, an opposite effect was observed at 915 MHz for all samples. Likewise, an inverse effect was observed for the penetration depth, with d_p values ranging from 31–35 mm to 17–22 mm at 915 MHz; which was lower at 2450 MHz, with values varying from 8–11 mm to 13–16 mm. This behaviour may be due to the thermal runaway phenomenon, in which the increase in temperature leads to an escalating increase in ε" and, consequently, a decrease in d_p, resulting in a very heterogeneous temperature distribution. The relative contributions of ionic conduction (C_σ") and dipolar rotation (C_d") mechanisms to the loss factor at 915 and 2450 MHz are shown in Fig. 4A,B. It can be seen that the dominant contribution to the loss factor depends on temperature and frequency. At low temperatures, the dipole rotation is the main loss factor mechanism for both frequencies; however, as the temperature increases, this contribution is replaced by the ionic conduction mechanism, achieving maximum values at 90 °C. This phenomenon was more pronounced at 915 MHz, with higher C_σ" values observed for semi-skimmed and skimmed milk.

3.6. Data analysis

3.6.1. Temperature-dependence effect on the dielectric properties

Table 2 exhibits the polynomial coefficients b_i ($i = 0, 1, 2$), coefficients of determination (R^2) and standard errors of estimate (δ_{est}) of the polynomial regression results of the temperature-dependence of electrical conductivity (σ) and dielectric properties (ε', ε", d_p, C_σ", C_d" and tanδ) for the commercial frequencies of 915 and 2450 MHz. All correlations were valid for the studied temperature range (5–90 °C). Electrical conductivity exhibited a linear correlation with temperature (0.993–0.995). Whole sheep milk showed the lowest angular coefficient (b_1) followed by semi-skimmed and skimmed milk. Whereas a higher fat content yields higher viscosity, the ions in whole milk may have less mobility than those in semi-skimmed and skimmed milk. Although ε' of all samples decreased almost linearly with temperature, a better fit was obtained with a quadratic correlation ($R^2 \geq 0.998$). A similar agreement was obtained for temperature-dependence of the quadratic equations of ε" and d_p. Similar results have been reported for other liquid foods (Franco et al., 2015; Siguemoto & Gut, 2016; Zhu et al., 2014, 2015b). The predicted ε' good agreement with experimental data for all types of milk and frequencies. The parameters ε", d_p and tanδ showed a better fit at 915 MHz, and for samples with higher fat content. Since the sum of C_σ" and C_d" is equal to 1, the estimated parameters were exactly the same values but with an inverse signal. A slight decrease in δ_{est} and R^2 for those

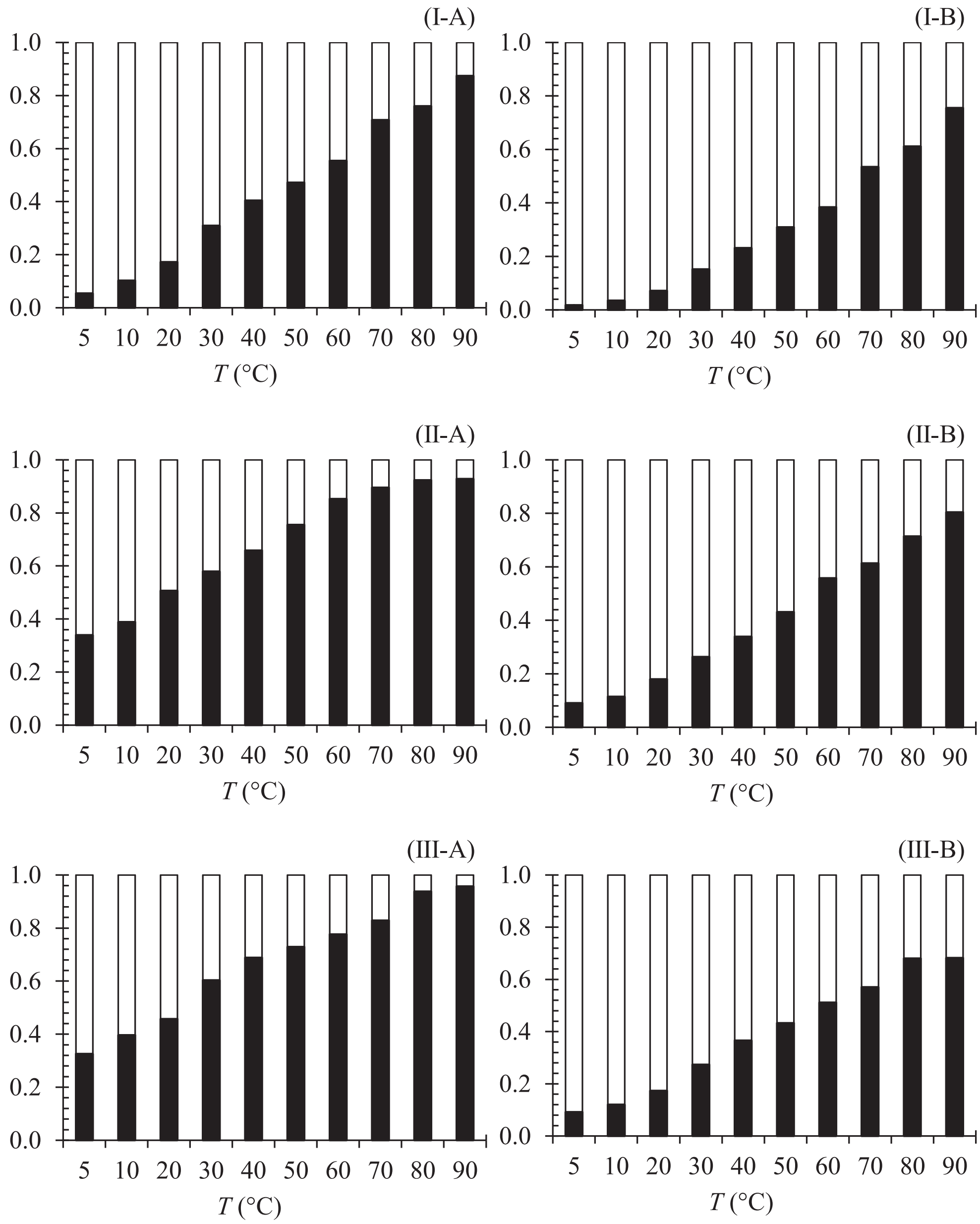

Fig. 4. Ionic (■; $C_\sigma'' = \varepsilon_\sigma''/\varepsilon''$) and dipole (□; $C_d'' = \varepsilon_d''/\varepsilon''$) contribution in the dielectric loss factor (ε'') of the whole (I), semi-skimmed (II) and skimmed (III) sheep milk at 915 MHz (A) and 2450 MHz (B).

parameters was observed with a decrease in fat content at 915 MHz.

3.6.2. Multivariable effect on the dielectric properties

MLR, PLSR and ANN were used as chemometric techniques to predict the dielectric properties (ε', ε'', d_p, C_σ'', C_d'' and tanδ) considering them as a function of both temperature and intrinsic characteristics of food, such as electrical conductivity, water activity, total soluble solids, titratable acidity, pH, colour, and moisture, ash, protein, fat, carbohydrates, and calcium contents.

MLR is one of the statistical methods aimed at modelling the relationship between two or more interpretive variables (independent) and a response variable (dependent) by fitting a linear correlation (Kavuncuoglu et al., 2018). MLR was performed by trial and error, considering only the significant parameters ($p < 0.05$). MLR achieved a good fit for all dielectric properties at both frequencies ($R^2 > 0.877$), except for tanδ at 2450 MHz ($R^2 = 0.496$). Water activity, L^*, TTA, pH, moisture, proteins, and carbohydrates were not significant for all correlations. Temperature showed a significant correlation for all dielectric properties, whereas electrical conductivity, a^*, b^*, ash, fat and calcium contents are presented in several correlations (Table 3). Higher average R^2 was found at 915 MHz ($R^2 = 0.980$), indicating this frequency might be a better choice than 2450 MHz ($R^2 = 0.872$) for predicting dielectric properties. The root mean square error (RMSE) was 0.334 and 0.450 for 915 and 2450 MHz, respectively (Table 4).

PLSR is a popular multivariate calibration algorithm in various fields, which seeks a fundamental relationship between the instrumental response X ($N \times P$) and the property of interest Y ($N \times 1$). The PLS regression coefficients were calculated by

Table 2
Adjusted polynomial parameters for temperature-dependence (5 °C ≤ T ≤ 90 °C) of electrical conductivity (σ) and dielectric properties, standard errors of estimate (δ_{est}), and coefficients of determination (R^2).[a]

Parameter	*f (MHz)*	Sheep milk	b_0	$b_1 \times 10^{-2}$ (°C^{-1})	$b_2 \times 10^{-4}$ (°C^{-2})	δ_{est}	R^2
σ (mS cm^{-1})	–	Whole	−0.2181	6.790	–	0.160	0.993
	–	Semi-skimmed	1.684	9.080	–	0.175	0.995
	–	Skimmed	1.859	10.17	–	0.237	0.993
ε'	915	Whole	54.53	−34.56	4.549	0.439	0.998
		Semi-skimmed	75.57	−45.07	15.36	0.375	0.998
		Skimmed	77.00	−27.89	2.044	0.233	0.999
	2450	Whole	50.15	−27.16	0.5203	0.317	0.998
		Semi-skimmed	70.49	−33.77	7.003	0.248	0.999
		Skimmed	71.66	−16.65	−5.950	0.201	0.999
ε”	915	Whole	10.96	2.002	1.270	0.088	0.991
		Semi-skimmed	12.85	3.374	5.749	0.286	0.988
		Skimmed	13.07	13.23	−3.344	0.399	0.983
	2450	Whole	13.82	−19.72	12.57	0.231	0.992
		Semi-skimmed	19.10	−25.97	16.34	0.503	0.978
		Skimmed	19.60	−23.60	15.89	0.403	0.980
d_p (mm)	915	Whole	35.32	−17.99	1.324	0.150	0.999
		Semi-skimmed	35.74	−23.93	4.449	0.397	0.995
		Skimmed	34.20	−30.19	13.00	0.430	0.994
	2450	Whole	9.277	22.04	−15.39	0.267	0.989
		Semi-skimmed	8.226	16.08	−9.016	0.373	0.975
		Skimmed	8.092	15.06	−10.82	0.251	0.978
C_σ”	915	Whole	0.0041	0.9528	0.0122	0.019	0.995
		Semi-skimmed	0.2677	1.272	−0.5744	0.018	0.993
		Skimmed	0.2804	1.102	−0.3923	0.023	0.988
	2450	Whole	−0.0072	0.3605	0.5378	0.013	0.997
		Semi-skimmed	0.0530	0.6021	0.3224	0.012	0.998
		Skimmed	0.0417	0.7766	0.0012	0.013	0.997
C_d”	915	Whole	0.9959	−0.9528	−0.0122	0.019	0.995
		Semi-skimmed	0.7323	−1.272	0.5744	0.018	0.993
		Skimmed	0.7196	−1.102	0.3923	0.023	0.988
	2450	Whole	1.007	−0.3605	−0.5378	0.013	0.997
		Semi-skimmed	0.9470	−0.6021	−0.3224	0.012	0.998
		Skimmed	0.9583	−0.7766	−0.0012	0.013	0.997
tanδ	915	Whole	0.2078	0.0886	0.2701	0.004	0.999
		Semi-skimmed	0.1719	0.1244	0.1850	0.006	0.995
		Skimmed	0.1697	0.2315	0.0460	0.006	0.995
	2450	Whole	0.2816	−0.3222	0.2972	0.004	0.974
		Semi-skimmed	0.2739	−0.2833	0.2134	0.007	0.935
		Skimmed	0.2756	−0.2946	0.2505	0.006	0.947

[a] Temperature polynomial correlations are calculated as: $y = b_0 + b_1T + b_2T^2$.

minimum least squares, between the scores and loadings from X matrix versus the scores from Y. PLSR was performed using the nonlinear iterative partial least squares (NIPLS) algorithm and the number of components or latent variables (LVs) was carefully chosen by cross-validation to reduce modelling subspace (Chen, Tan, Lin, & Wu, 2018). The PLSR model of 915 MHz data set generated 4 components, with Q^2_{acum} (a measure of cross-validation of the PLS model), R^2 X_{acum} (percentage variability explained in 3 dimensions of the dependent variable), and R^2 Y_{acum} (percentage variability explained in 3 dimensions of the independent variable) values of 0.913, 0.927, and 0.965, respectively. However, lower values were obtained by the PLSR model of 2450 MHz dataset with 4 components (Q^2_{acum} = 0.797, R^2 X_{acum} = 0.923, and R^2 Y_{acum} = 0.868). Those values indicate a good predictive ability of the models and explanation of the variability of the data, probably due to low heterogeneity of the milk samples. A better fit was obtained at 915 MHz (R^2 = 0.966; RMSE = 0.453) than at 2450 MHz (R^2 = 0.869; RMSE = 0.519), which is in accordance with the previous MLR results (Table 4). The variables considered more important in predicting the dielectric properties were temperature, electrical conductivity, and a^* and calcium content.

ANNs are generally computing systems that imitate some properties of biological neurons. Input layer, hidden layer(s), and output layer of neurons are the main parts of each ANN structure. Neurons in the input layers receive the input data and normalise and transmit them to the hidden layer. A linear combination of the outputs from all neurons in the previous layer is calculated by every neuron of a subsequent layer and then bias (weight values associated with individual nodes) is added. Neurons in the hidden layers apply a specific non-linear function (transfer function) to the collection of linear combination and bias, and finally give the predicted models for the output (Torkashvand, Ahmadi, & Nikravesh, 2017). The best ANN architectures for 915 and 2450 MHz datasets were obtained using 14 input neurons and 6 output neurons. The input neurons were composed of temperature, electrical conductivity, water activity, colour (L^*, a^* and b^*), titratable acidity, pH, and moisture, ash, protein, fat, carbohydrates, and calcium contents. Output neurons correspond to the dielectric properties (ε', ε”, d_p, C_σ”, C_d” and tanδ). The hidden layer was comprised of 5 and 6 neurons for 915 and 2450 MHz, respectively. According to Table 4, determination coefficients obtained for all dielectric properties were at least higher than 0.992. For both datasets average R^2 was 0.997. ANN model achieved the best agreement among the chemometric techniques tested, reaching RMSE of 0.216 and 0.142 for 915 and 2450 MHz, respectively. Sensitivity analysis showed calcium content, electrical conductivity, b^*, a^*, moisture and temperature as the most important variables.

3.6.3. Pattern recognition of milk type

LDA, SIMCA, k-NN, and ANN were used as pattern recognition tools to predict the type of milk (whole, semi-skimmed, and skimmed). Models were evaluated for their discriminative power,

Table 3
Parameters of the multiple linear regression (MLR), standard errors of estimate (δ_{est}), and coefficients of determination (R^2) for the operational conditions, milk physicochemical attributes and composition dependence of the dielectric properties.[a].

X_i	b_i	MLR											
		915 MHz						2450 MHz					
		ε'	ε"	d_p (mm)	C_σ"	C_d"	tanδ	ε'	ε"	d_p (mm)	C_σ"	C_d"	tanδ
Constant	b_0	−214	39.6	12.7	−8.92	9.92	0.474	−223	−3.35	−0.940	−2.634	3.634	0.583
T	b_1	−0.782	−0.713	−0.668	1.29	−1.29	1.26	−0.728	−0.738	1.58	0.973	−0.973	−0.683
σ	b_2	0.213	1.60	−0.349	−0.487	0.487	−0.356	0.204	–	−0.974	–	–	–
a_w	b_3	–	–	–	–	–	–	–	–	–	–	–	–
L^*	b_4	–	–	–	–	–	–	–	–	–	–	–	–
a^*	b_5	–	–	–	–	–	–	–	−0.492	–	–	–	–
b^*	b_6	–	−0.186	0.084	–	–	−0.070	–	–	0.100	0.057	−0.057	–
TTA	b_7	–	–	–	–	–	–	–	–	–	–	–	–
pH	b_8	–	–	–	–	–	–	–	–	–	–	–	–
Moisture	b_9	–	–	–	–	–	–	–	–	–	–	–	–
Ash	b_{10}	–	–	–	–	–	–	–	0.128	–	–	–	–
Proteins	b_{11}	–	–	–	–	–	–	–	–	–	–	–	–
Fat	b_{12}	–	0.109	–	–	–	–	–	–	–	–	–	–
Carbohydrates	b_{13}	–	–	–	–	–	–	–	–	–	–	–	–
Calcium	b_{14}	0.405	–	–	0.752	−0.752	–	0.438	–	–	0.167	−0.167	–
R^2		0.996	0.983	0.984	0.975	0.975	0.971	0.997	0.896	0.877	0.984	0.984	0.496
δ_{est}		0.857	0.437	0.681	0.041	0.041	0.015	0.688	1.134	0.869	0.311	0.311	0.019

[a] Dielectric properties: relative electrical permittivity (ε'); dielectric loss factor (ε"); penetration depth (d_p); percent contribution of ionic conduction (C_σ") and dipole rotation (C_d") mechanisms; loss tangent (tanδ = ε"/ε'). Equations were calculated as: $y = b_0 + \Sigma^n{}_{i=1}\, b_i X_i$.

Table 4
Performance analysis of the chemometric tools expressed by coefficient of determination and root mean square error for the prediction of the dielectric properties according to operating parameters and milk characteristics.[a]

Chemometric tool	f (MHz)	ε'	ε"	d_p(mm)	C_σ"	C_d"	tanδ	Average
Coefficient of determination (R^2)								
MLR	915	0.996	0.983	0.984	0.975	0.975	0.971	0.980
	2450	0.997	0.896	0.877	0.984	0.984	0.496	0.872
PLSR	915	0.993	0.947	0.984	0.955	0.955	0.960	0.966
	2450	0.994	0.899	0.847	0.980	0.980	0.512	0.869
ANN	915	0.997	0.995	0.996	0.998	0.998	0.996	0.997
	2450	0.999	0.997	0.994	0.999	0.999	0.992	0.997
Root mean square error (RMSE)								
MLR	915	0.823	0.424	0.661	0.040	0.040	0.015	0.334
	2450	0.661	1.109	0.849	0.030	0.030	0.018	0.450
PLSR	915	1.158	0.764	0.668	0.054	0.054	0.018	0.453
	2450	0.952	1.112	0.964	0.035	0.035	0.018	0.519
ANN	915	0.673	0.239	0.353	0.012	0.011	0.006	0.216
	2450	0.453	0.183	0.196	0.008	0.008	0.002	0.142

[a] Abbreviations are: MLR, multiple linear regression; PLSR, partial least square regression; ANN, artificial neural network. Dielectric properties are: relative electrical permittivity (ε'); dielectric loss factor (ε"); penetration depth (d_p); percent contribution of ionic conduction (C_σ") and dipole rotation (C_d") mechanisms; loss tangent (tanδ = ε"/ε').

and sensitivity analysis was used to determine the most important variables for each technique. LDA is a supervised pattern recognition technique based on a linear function, which is used for linear classification or leads to dimensionality reduction (Efenberger-Szmechtyk, Nowak, & Dorota-Kregiel, 2018). LDA showed great results with 100% of recognition and prediction capacity for both datasets. SIMCA is a supervised classification technique that uses samples with a known origin (training samples) to derive a classification rule, which allows classifying new samples (test samples) with unknown origin into one of the classes, according to the characteristics of the new samples. SIMCA considers different classes that are modelled individually by a separate principal component (PC) model. The optimal number of PCs is determined for each class, using a cross-validation procedure (Mees et al., 2018). SIMCA was also used to perform the discrimination among the three types of milk, according to the skimming process. Each type of milk was modelled separately by selecting an appropriate number of PCs necessary to describe each class. The SIMCA models for both datasets 915 and 2450 MHz also exhibited 100% correct recognition and prediction capacity. Although all variables exhibited similar influence on the construction of the model, the variables ε', C_σ" and C_d" showed greater discriminative power among the classes. The k-NN classifier is a non-parametric and instance-based learning algorithm that aims to classify unlabelled observations by assigning them to the class of the most similar labelled examples. Characteristics of observations are collected for both training and test dataset (Zhang, 2016). The k-NN model showed a better fit for 3-nearest neighbours, with recognition and prediction percentages of 100% for both datasets, including all three classes. ANN was also performed as a pattern recognition technique to predict the type of sheep milk based on the dielectric properties. ANN models of both frequencies comprised 6 input neurons (ε', ε", d_p, C_σ", C_d" and tanδ), 3 hidden neurons, and 3 output neurons (whole, semi-skimmed, and skimmed classes), using logistic and identity functions in hidden and output layers, respectively. The confusion matrix showed the correct prediction percentage of 100% for all datasets (i.e., 100% recognition capacity and 100% prediction capacity for training and test sets, respectively, for whole, semi-skimmed and skimmed sheep milk using LDA, SIMCA, k-NN and ANN). In general, the sensitivity analysis showed ε' and C_d" as the most discriminative variables to the model, followed by d_p, tanδ, ε" and C_σ". Therefore, all chemometric methods presented interesting findings and could be an effective quality control tool in sheep milk processing, with practical utility for the variables of the microwave processing.

4. Conclusions

All chemometric techniques showed a great capacity to predict the dielectric properties. The ANN model had the best performance but MLR and PLSR were more suitable models due to the lower number of coefficients to be adjusted. Generally, a better fit and more significant parameters were found at 915 MHz than 2450 MHz, indicating that 915 MHz might be a better choice for predicting dielectric properties. Generally, sensitivity analysis showed temperature, electrical conductivity, b^* and calcium content as the variables with the most impact on the prediction of dielectric properties. These results may be useful for further studies of process simulation and economic feasibility of microwave heating of sheep milk. All pattern recognition techniques have shown 100% of recognition and prediction ability to classify correctly the type of milk. The potential application of fast measurements, such as dielectric properties, associated with chemometric tools to determine the type of milk can be a very promising approach to monitor quality parameters in the milk processing and adulteration in the dairy industry.

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